thought to be mesenchymal-like cells that, having undergone an epithelial-to-mesenchymal transition, are more likely to migrate. However, cancer cells may also show amoeboid-like motility when mesenchymal-like movement is blocked (Wolf and Friedl, 2006). Rac is required in the mesenchymal mode of motility but not apparently in the amoeboid mode. The authors found that Rab5 is also required for mesenchymal motility, implying that Rab5-regulated trafficking of Rac is involved in this type of movement. Palamidessi et al. propose that Rab5 can control conversion between amoeboid and mesenchymal migration. Expression of Rab5 in cultured melanoma cells, a model for amoeboid motility, induced a change from amoeboid to a more mesenchymal-like morphology and movement. Conversely, inhibition of Rab5 in cultured colon carcinoma cells, a model for mesenchymal motility, resulted in a switch to a more amoeboid-like morphology and motility. Most intriguingly, Rab5 seems to also play a role in normal cell migration because the authors observed a requirement for Rab5 in vivo in the guidance of primordial germ cell migration during zebrafish development.

The work by Palamidessi and colleagues establishes a requirement for Rab5-dependent endocytic trafficking in Rac activation and raises many new questions. For example, do amoeboid and mesenchymal migration behaviors correlate with the ability to induce CDRs after RTK activation? Also, a previous study showed that in response to a growth factor gradient, CDRs form in a polarized fashion toward the stimulus and generate a tubovesicular network (Orth et al., 2006). Could a Rab5-RacGTP endomembrane system activated by receptors internalized at CDRs be the localized secretory pathway that delivers actin-associated proteins to the cellular protrusions? Clearly, there is much yet to learn about the induction of this Rac-Rab5 trafficking circuit and how it may control the balance between growth factor signaling and cell shape, migration, and polarity.

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Deconstructing Export of Malaria Proteins

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The virulence of the malaria parasite *Plasmodium falciparum* is mediated by parasite proteins exported to the surface of infected erythrocytes. In this issue, Maier et al. (2008) report a screen of malaria parasite genes predicted to be involved in parasite protein export and trafficking within the host erythrocyte and discover that many more than expected are essential for parasite survival in vitro.

Plasmodium falciparum, the protozoan parasite that causes the most deadly form of malaria, provides one of the most sophisticated examples of host cell manipulation by an intracellular pathogen. This parasite is a unicellular eukaryote that invades host erythrocytes and resides within a parasitophorous vacuole. It profoundly alters the host cell (Figure 1), for example, rendering the host cell rigid and spherical. Within the cell, *P. falciparum* induces the formation of membrane-bound organelles, including a tubulo-vesicular network linked to the parasitophorous vacuole as well as flat lamellar membranes located beneath the erythrocyte plasma membrane called Maurer's clefts. A variety of parasite proteins are exported from the parasitophorous vacuole into the host cell milieu. Some of these proteins are expressed at the host cell surface, including PfEMP1 (*P. falciparum* erythrocyte membrane protein 1), which is displayed on parasite-induced protuberances called knobs (see SnapShot by A. Scherf et al. in this issue). PfEMP1 enables infected erythrocytes to bind to endothelial cells of host capillaries, leading to their sequestration in the peripheral vasculature and thus

preventing their clearance by the host spleen. Binding of infected erythrocytes to the endothelial cells of brain capillaries causes a fatal form of malaria (cerebral malaria). In this issue, Maier et al. (2008) take gene targeting in *P. falciparum* to a new level by combining a large-scale gene knockout strategy with functional characterization of more than 80 parasite proteins that are potentially exported out of the parasitophorous vacuole into the host erythrocyte.

PfEMP1 belongs to an antigenically diverse family of proteins. The parasite expresses only one form of PfEMP1 on the erythrocyte surface at any one time but can switch to a different form to escape the host antibody response (Ralph and Scherf, 2005). However, the molecular basis of PfEMP1 export from the parasitophorous vacuole to the host cell surface is still poorly understood (Charpian and Przyborski, 2008). The identification of a short peptide sequence—PEXEL (*Plasmodium* export element; Marti et al., 2004), also called HT (host targeting; Hiller et al., 2004)—in malaria parasite proteins that pass out of the parasitophorous vacuole has enabled the prediction of the complete set of proteins that are exported into the erythrocyte (exportome). The most expanded version of the *P. falciparum* exportome (Sargeant et al., 2006; van Ooij et al., 2008) consisting of 420 to 450 proteins includes 256 members of paralogous protein families specific to *P. falciparum*, of which 59 are PfEMP1 adhesins. Although translocation across the parasitophorous vacuole membrane via a PEXEL motif is functionally conserved across *Plasmodium* species, the *P. falciparum* exportome is 5–10 times larger than that of other malaria parasites, reflecting the unique pathogenicity of *P. falciparum*, namely its ability to become sequestered in host capillaries.

To gain insights into how PfEMP1 reaches the erythrocyte surface, Maier and colleagues introduced loss-of-function mutations into 46 *P. falciparum* genes encoding proteins containing the PEXEL sequence, 5 genes encoding PEXEL-deficient products that are nonetheless exported to the erythrocyte surface, and 32 genes encoding proteins with a signal sequence but no PEXEL motif. The mutations were introduced in a cultured parasite line that stably expresses a PfEMP1

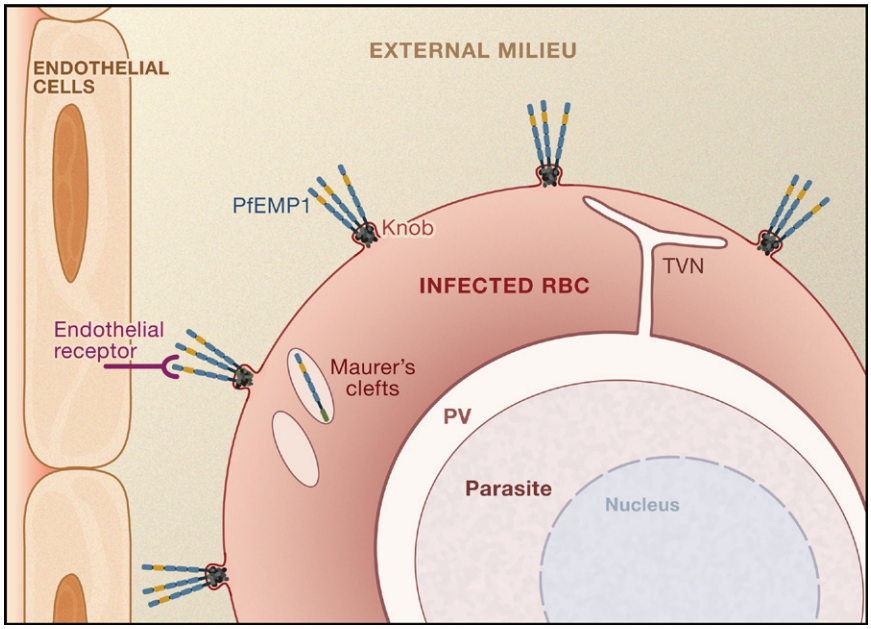

Figure 1. The Malaria Parasite Remodels the Host Erythrocyte

After invading the host red blood cell (RBC), the malaria parasite *Plasmodium falciparum* resides within a parasitophorous vacuole (PV) and induces extensive remodeling of the host erythrocyte. For example, *P. falciparum* induces the formation of a tubulo-vesicular network (TVN) linked to the PV, as well as flat lamellar membranes called Maurer's clefts that lie below the erythrocyte plasma membrane. The parasite also directs the formation of molecular protuberances (knobs) at the host cell surface that display parasite surface proteins such as PfEMP1, which allow infected erythrocytes to adhere to host endothelial cells through interaction with endothelial receptors.

protein encoded by the *var2csa* gene, which enables infected erythrocytes to bind to chondroitin sulfate A (CSA). Of 83 transfections, 53 (64%) generated knockout parasites, which were then tested in human erythrocytes in vitro for trafficking and surface expression of PfEMP1, binding to CSA, knob assembly, and the ability to induce erythrocyte rigidity. With these assays, the authors uncovered many mutant parasites that showed defects in distinct steps in the PfEMP1 transport process. For example, in six mutants PfEMP1 was not expressed at the erythrocyte surface: instead, the protein was blocked in the parasitophorous vacuole in three mutants, and it did not move beyond the Maurer's clefts in the other three mutants. Two mutants showed defects in knob formation even though they both presented PfEMP1 normally on the erythrocyte surface: one formed only rudimentary knobs with abnormal distribution of the knob-associated histidine-rich protein (KAHRP), and the other failed to form knobs altogether and did not transfer KAHRP from the Maurer's clefts to the erythrocyte membrane. Most of the parasite mutations caused only small alterations in the pathogen-induced deformation of infected erythrocytes, suggesting that many gene products play a role in the rigidity phenotype of infected host cells. However, several mutants did cause markedly reduced rigidity in their host erythrocytes. Unexpectedly, four mutants conferred more rigidity on the host cell membrane than wild-type parasites. Of these, three mutants showed normal adherence to CSA, whereas one lacked PfEMP1 on the cell surface. This collection of mutants with such different phenotypes will be a valuable resource for deconstructing the PfEMP1 export pathway and for elucidating the molecular basis of knob formation and host cell deformation. These parasite mutants may also prove useful in the search for new drugs to block adherence of infected erythrocytes to host capillaries.

One surprising result is that 23% of the genes encoding proteins that contain the PEXEL motif were found to be essential for survival—parasites harboring mutations in these genes could not be isolated. These findings contrast with the expecta-

tion that because selection of the knockout *P. falciparum* lines took place in vitro, failure of processes involved in evading the host immune system (such as the correct display of PfEMP1 on the host erythrocyte surface) would not be detrimental for growth in vitro. Of course, the exportome is not just important for enabling infected erythrocytes to adhere to endothelial cells and to evade immune destruction. Indeed, it has long been appreciated that the parasite must also acquire essential nutrients from the external milieu, which requires the tubulo-vesicular network and the expression of transporter proteins in the erythrocyte membrane (Lauer et al., 1997). The parasite may also need to remodel the structure of the host erythrocyte and increase the stability of the erythrocyte membrane to allow for expansion as the parasite grows (and possibly also to block invasion by new parasites) (Pei et al., 2007). One would predict that essential genes mediating parasite growth and metabolic functions would be conserved across *Plasmodium* species. Indeed, a portion of the *P. falciparum* exportome is conserved in a variety of *Plasmodium* species infecting humans and rodents. However, this "conserved core" of the *Plasmodium* exportome consists of only 10 to 30 components, depending on the prediction algorithms (van Ooij et al., 2008). Given the small number of conserved core genes, why did Maier et al. discover so many *P. falciparum* exportome genes that are essential for parasite survival in vitro? The failure to recover particular mutant parasites may reflect the poor efficiency of *P. falciparum* transfection, but a more interesting possibility is that these "essential" genes reflect more complex metabolic requirements for the intraerythrocytic replication of *P. falciparum* relative to other malaria parasites. Clearly, more work is needed to better define the exportome proteins that are vital for parasite replication within the host erythrocyte and for preventing parasite destruction in vivo. The valuable study by Maier et al. provides a useful toolbox to start obtaining answers to these questions.

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The Mitochondrial Proteome: From Inventory to Function

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Mitochondria are central to cellular energetics, metabolism, and signaling. In this issue, Pagliarini et al. (2008) report the largest compendium of mammalian mitochondrial proteins to date. Together with proteomic studies in yeast, this study represents an important step toward the systematic characterization of the mitochondrial proteome and of mitochondrial diseases.

Mitochondria are cellular powerhouses, synthesizing the bulk of the ATP used by eukaryotic cells. The cellular function of mitochondria, however, is not limited to bioenergetics. They play crucial roles in the metabolism of amino acids and lipids, the biosynthesis of heme and iron-sulfur clusters, cell signaling, and apoptosis. Mitochondrial proteins are encoded by two genomes. In humans, the mitochondrial genome codes for only 13 proteins; the remaining 99% of mitochondrial proteins are encoded by nuclear genes and are synthesized as precursors on cytosolic ribosomes and then are imported into mitochondria (Bolender et al., 2008). To understand the role of mitochondria in health and disease, it is important to know the protein composition of this organelle. In this issue, Pagliarini et al. (2008) now report a major step in compiling a comprehensive compendium of mammalian mitochondrial proteins (the MitoCarta).